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Plasma Pepsinogen Test for Hypobiotic Nematode Larvae in Abomasal Glands of Sheep

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PLASMA PEPSINOGEN TEST FOR HYPOBIOTIC NEMATODE LARVAE IN ABOMASAL GLANDS OF SHEEP

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

It has been long recognized that gastrointestinal parasites are a burden to sheep and other ruminants. As early as the turn of the century, Ransom (1908) reported that stomach worms were such a problem to sheep producers that the need for a solution was critical. Much progress has been made in the control of ruminant parasites, but the problems of the past are present even today.

Today's agricultural economy is burdened with losses due to ruminant internal parasites. The United States Department of Agriculture (1980) reported an annual loss of $98 million to the sheep industry due to parasitism. They estimated the 12.5 million head population to be worth $975 million, so this means up to a 10 percent loss. They further divided this loss into 2 catetories: 1. Direct loss--death, illness, decreased weight gains, and carcass or organ condemnation at the slaughter-house. 2. Indirect loss--decreased feed efficiency, anorexia, delay in attainment of breeding age, reduced reproductive efficiency, and increased susceptibility to other diseases.

The American Association of Veterinary Parasitologists (1983) reported that nematodes in sheep represent an unquestionably great production loss, and that the absence of deworming on a routine basis would hamper modern sheep production.

Dunn (1978) states that trichostrongylidosis is the most important of all diseases affecting the ovine alimentary tract. These trichostrongylid nematodes include Haemonchus contortus, Ostertagia circumcincta, O. trifurcata, and Trichostrongylus axei. Haemonchus and

Ostertagia are especially important because they damage the abomasal mucosa. After ingestion, the infective third stage larva, or L_3, exsheaths and burrows into the digestive glands, and becomes a fourth stage larva, or L_4. According to Armour (1974), 18 to 21 days after L_3 ingestion occurs, the nematode reaches sexual maturity. However, while in the gastric glands, these L_4 invade the parietal, or hydrochloric acid (HCl) secreting cells, damaging them, and replacing them with non-acid secreting cells. This leads to an increase in abomasal pH, and further problems: 1. Reduced pepsinogen activation to pepsin. 2. Failure to denature proteins. 3. Loss of "bacteriostatic effect."

Armour (1974) also describes the subsequent permeability increase of the abomasal wall to pepsinogen. A loss of integrity of the cell junctions in the damaged areas is the route of exit that can lead to elevated plasma pepsinogen levels.

Dunn (1978) further points out that as a part of their normal, direct life cycle, the trichostrongylids use hypobiosis, or "arrested larval development" as an evolved survival mechanism. During periods of adverse climate, they merely halt at the L_4 stage to overwinter in the abomasal glands. According to Armour (1974), they resume development in the spring and reach sexual maturity within 14 to 17 days. The sudden presence of great numbers of mature nematodes is immediately followed by the presence of increased fecal egg counts. This phenomenon has acquired the name "spring rise."

Armour (1974) has suggested that the "spring rise" may be analogous to diapause in insects. Diapause is described by Highnam and Hill

(1977) as being under hormonal control, but research in nematodes is hampered by anatomical dissimilarities as compared to insects.

Recent tests for plasma pepsinogen levels have been implemented by researchers such as Armour (1974) and Thomas and Waller (1975) in an attempt to correlate the damage done to the abomasal mucosa by these nematodes in normal-seasonal, and in long-term arrested development. Another practical application being pursued is to find a correlation between plasma pepsinogen levels and fecal egg counts.

Campbell, et al. (1983) describe the development of a strong new antiparasitic compound, Ivermectin, by MSD AGVET division of Merck and Co., Inc., Rahway, New Jersey, that is effective against these inhibited L_4 trichostrongylid stages, especially Ostertagia. This has made the compound of great interest in this study.

Levamisole HCl, manufactured by Pitman-Moore, Inc., Washington Crossing, New Jersey, was also used in one test to observe any increase in rate of weight gain.

The primary objectives of this research were:

1. An evaluation of the advantages of worming based upon any significant increases in rate of weight gain in two different groups of lambs under quite different management conditions.

2. The inspection, at the slaughter of random test animals, of abomasal contents to determine nematode genera present.

3. The testing for plasma pepsinogen levels in blood samples according to the method outlined by Black (1973), and the correlation of these to fecal egg counts.

4. The observation of the "spring rise" phenomenon, and an attempt to correlate it to various environmental conditions that may be related to it.

REVIEW OF LITERATURE

Superfamily Trichostronglyoidea

Family Trichostronglidae

Life Cycle

All of the nematodes of the family Trichostrongylidae have a direct life cycle. Ivens, Mark and Levine (1978) write that the eggs are passed in the feces, and within 2 weeks after hatching the larvae undergo 2 molts to progress from the L_1, or first stage larva, to the L_3, or infective stage. The L_3 is infective when ingested, but can remain infective in the soil and vegetation for months. After ingestion, the larvae exsheath, become L_4, and enter the gastric pits for a period of "arrested development" as described by Armour (1974). The L_4 then returns to the lumen in a few weeks, goes through 2 more molts, becomes sexually mature, and the female begins to lay her eggs.

Haemonchus contortus

Haemonchus is referred to by many common names: the large stomach worm, barberpole worm, twisted stomach worm, and the wireworm. Honess and Winter (1956) point out that many of its names are derived from the fact that the female has white ovaries spirally wound around the straight red intestine, giving a barberpole appearance.

These trichostrongylids are blood suckers, and according to Corwin and Brauer (1984), the early L_4 invades the mucosal lining of the abomasum. Miller (1974) calls them "the most destructive South Dakota

sheep parasite." This, he writes, is due to the fact that the female can lay up to 6000 eggs daily, and a heavily infested animal can yield up to 3 million eggs in a single day.

Even though they can be a prolific parasite, Malone (1983) describes the pre-infective larvae, L_1 and L_2, and the eggs as being susceptible to adverse weather conditions. Dunn (1978) writes that the Haemonchus eggshell is more permeable to water than any of the other trichostrongylids, and will desiccate faster in dry conditions than its close relatives. This means that only the L_3 stage of Haemonchus is resistant. Trichostrongylus, on the other hand, has two resistant stages: L_1 in the egg and L_3.

Conversely, Dunn (1978) writes that Haemonchus eggs can be extremely successful on wet pastures, and that the most highly infected areas appear to be the mid-west and south-west in the U.S.

Dunn (1978) also writes that Haemonchus can go hypobiotic to a greater extent than any of the other trichostrongylids, and tends to do so earlier than does Ostertagia. He describes hypobiosis, or "arrested development," as an evolved mechanism for use by species that are not well adapted to drastic climatic changes. He also dispels the notion that the "spring rise" is caused solely by decreased resistance associated with parturition. It is also due to the great number of trichostrongylids coming out of "arrested development."

Structural Features

As previously stated, the female of Haemonchus is primarily

distinguishable by her white ovaries that wind around her red intestine in barberpole fashion. Another distinguishing feature of the female, according to Whitlock (1960), is a large vulvar flap located anterior to the anus. Morgan and Hawkins (1949) write that the female is 18 to 30 mm long, 500 microns wide, and has a sharply pointed tail.

They describe the male as being smaller at 10 to 20 mm long and 400 microns wide. It has a small buccal cavity containing a dorsal lancet, or "blood tooth." It also has two large, spine-like cervical papillae, and two barbed spicules 300 to 600 microns long, each having a ball tip. The most outstanding feature Dunn (1978) describes is the prominent bursa, and upon microscopic examination, the asymmetrically placed bifurcate dorsal ray.

Egg Features

Trichostrongylid egg identification is difficult due to morphological similarities among the genera. Ivens, Mark and Levine (1978) write that all eggs are ellipsoidal and smooth, have 8 to 32 cells when passed in the feces, and are 80 to 100 by 40 to 50 micrometers. Thienpont, Rochette, and Vanparijs (1979) describe Haemonchus eggs as being more regular, with flattened, wide poles.

Georgi (1969) concurs with the difficulty of egg identification: "Except for Nematodirus and Marshallagia, the generic identity of individual strongyle eggs cannot be reliably established by microscopic inspection or by micrometry."

Ostertagia circumcincta and O. trifurcata

The common name for these trichostrongylids are the medium or brown stomach worm. Corwin and Brauer (1984) write that they also damage the gastric mucosa, causing mucosal hypertrophy, giving it the look of "Morocco leather." Dunn (1978) states that their prevalence in the northern hemisphere is between 7 and 12 to 1 in favor of O. circumcincta over O. trifurcata. He also says that Ostertagia has been the center of intensive study in recent years. Armour (1974) and Thomas (1975) have used them in their research to evaluate their relationship to plasma pepsinogen levels in infected ruminants.

Chiejina and Clegg (1978) have analyzed the epidemiology of ostertagiasis in calves, and say this about the weather's role:

> In those years with unusually dry summers the incidence of the disease differs from the usual pattern. In such years the herbage infection is low during the summer but very high in late autumn and winter when the faecal pats, which act as reservoirs of the infective larvae during the dry period, disintegrate with onset of wet weather, releasing large numbers of larvae.

Structural Features

Dunn (1978) describes Ostertagia as small, slender worms, with a reddish-brown color. They have no buccal capsule, but both sexes do have a tiny pair of cervical papillae. The male is 7 to 9 mm long, while the female is larger at 8 to 12 mm. The male spicules have 3 distinguishable branches, and the terminal portion of the main branch forms the spicule body. Morgan and Hawkins (1949) describe the male bursa as becoming 3 times the width of the body when it is spread out.

Dunn (1978) describes the female vulvar flap as being unique to different Ostertagia species, and therefore a key to correct identification. The tip of the tail can have 5 or 6 rings, which is where O. circumcincta derived its name. Morgan and Hawkins (1949) write that the eggs measure 85 to 103 microns in length and 44 to 56 microns in width, with the 2 sides of the eggshell being curved. Thienpont, Rochette, and Vanparijs (1979) call them a regular ellipse, symmetrical, with narrow poles. They also describe them as being less rounded than Haemonchus eggs.

Trichostrongylus axei

Trichostrongylus is referred by Ivens, Mark and Levine (1978) as the brown stomach worm. They also write that due to its smaller size versus Haemonchus, it is less pathogenic when present in equal numbers. Corwin and Brauer (1984) state that the adults can consume mucosal tissue fluids.

Structural Features

Dunn (1978) describes the adults as small and hair-like. The males vary from 5.5 to 7 mm long and the females are 6 to 8 mm long. They have no obvious buccal capsule when viewed microscopically, and the bursa has a "ventro-ventral ray" set apart from the others. The females are small, have double ovijectors, and lack accessory structures in the head and vulvar regions. Morgan and Hawkins (1949) describe the spicules as dissimilar and the ovijectors as less well developed as compared to other genera.

Egg Features

Thienpont, Rochette, and Vanparijs (1979) call the eggs medium-sized. They are about 86 to 40 microns, and are an irregular ellipse. Their poles are narrow, one being more rounded than the other, and one side wall is flattened.

Hypobiosis, Spring Rise, and Diapause

Campbell et al. (1983) describe hypobiotic larvae--L_4--as important pathogens that are resistant to most antiparasitic compounds, one exception being the new compound Ivermectin by Merck and Co. Thomas and Waller (1975) refer to the emergence of Ostertagia as constituting "Type II" ostertagiasis; this they contrast with "Type I" ostertagiasis, where the animals affected are merely ingesting infective larvae from the pasture. These infective larvae, according to Anderson et al. (1965) are ingested in late autumn, and may stay dormant in the gastric pits of the abomasum for up to 6 months. They also write that the mechanism of the autumn-inhibition relationship is not fully understood or agreed upon.

Armour (1974) defines the "seasonal inhibition" of trichostrongylids as a form of evolved diapause, and it allows them to survive adverse climatic conditions, especially low temperatures. Highnam and Hill (1977) define diapause as "a state of arrested development." They write that in insects it may occur in the egg, larvae, or adult stages, but usually only one diapause occurs in the life-cycle. In insects it is characterized by decreased metabolism, excess fat stores, and resistance to cold and desiccation. They further describe 2 types of diapause: 1. Obligatory--occurring at a specific time regardless of environmental conditions. 2. Faculative-forced upon the organism by the presence of environmental conditions. They describe the mechanism for diapause in post-embryonic insects as a temporary halt in activity within certain areas of the endocrine system, which control growth and

development. Hickman et al. (1979) state:

> Diapause always occurs at the end of an active growth stage of the molting cycle so that, when the diapause period is over, the insect is ready for another molt.

Highnam and Hill (1977) describe nematodes as difficult to work with experimentally, but state that their endocrine systems may also be involved in diapause. In nematodes, they conclude, molting is similar to that in arthropods as proven experimentally by inducing nematode molting with insect hormones.

Anderson et al. (1965) describe the damage done by hypobiotic larvae as leading to the replacement of active parietal cells with mucous-type epithelium. Further damage can lead to epithelial cell sloughing with the formation of "thumb-print" lesions.

Dunn (1978) uses the term "peri-parturient rise" when referring to the increase in worm egg numbers in ewe feces in the spring. He relates this to a decrease in resistance to parasites during lambing season, but also to the time of year: spring weather's arrival coincides with lambing. Whatever these environmental stimuli--if indeed they are factors--they appear to arouse inhibited L_4 trichostrongylid larvae to resume their development and to initiate egg production in the spring.

It must be realized that the removal of inhibited larvae from the affected animal will not necessarily eliminate the worm burden. Johnstone (1985) writes that if animals are kept on the same pasture following treatment, they will be reexposed to infective larvae, and that parasite control programs that ignore this are "doomed to failure."

Plasma Pepsinogen

Undoubtedly, the most interesting aspect of this entire study was the attempt to correlate increased plasma pepsinogen levels with actual L_4 burdens in wintering test animals, based on fecal egg counts. Studies in humans have been carried out for many years that observe uropepsinogen levels in gastric ulcer patients, as outlined by Edwards, Jepson, and Wood (1960). A more accurate method detailed by these same authors for determining pepsinogen levels in the body was the introduction of plasma pepsinogen values, taken from venous blood. They recorded plasma pepsinogen levels as the amount of tyrosine released from a 1 ml sample of plasma that had been incubated at 37°C for 24 hours. Harvey-White and Allen (1982) describe the process as

> the acid activation of pepsinogen to pepsin followed by measurement of the amount of tyrosine units released during the pepsin digestion of hemoglobin substrate, or of serum proteins.

Harvey-White et al. (1983) feel that the L_4 emergence from the gastric mucosa causes elevated pepsinogen levels. This is in addition to damaged parietal cells as cited earlier. Regardless, pepsinogen, the precursor of the digestive enzyme pepsin, must come in contact with an acid environment in order to be converted into the active proteolytic enzyme state: Pepsin. Vander, Sherman, and Luciano (1980) describe this as occurring via the acid breaking off a small fragment from the pepsinogen molecule, causing the protein to change its shape and expose the active site. Pepsin then splits specific peptide bonds, a step crucial to normal protein metabolism.

Plasma pepsinogen values are expressed in milliunits (mu) of

tyrosine released, as explained by Thomas and Waller (1975), and are considered to be a practical guide to abomasal damage. Levels of worm-free lambs in one test cited by these authors varied from 175 to 350 mu, with a high of 2600 mu. Selman, Armour, and Jennings (1977) corroborate this by reporting that their research in Great Britain indicated levels above 3000 mu in cattle were indicative of significant burdens with Ostertagia.

All researchers are not in total agreement as to the confidence of the plasma pepsinogen test. Thomas and Waller (1975) pointed out the results of a test which showed a good relationship between elevated plasma pepsinogen levels, and Type II ostertagiasis in cattle, and contrasted it with another test where there was no correlation between fecal egg counts and pepsinogen levels. They further write that the correlation between plasma pepsinogen levels and recent larval intake, as is common in Type I ostertagiasis, is more correct.

Ivermectin

Campbell et al. (1983) describe Ivermectin as one member of a newly discovered class of drugs: the avermectins. They were discovered in the fermentation broth of an actinomycete culture, obtained from the Kitasato Institute of Japan. The actinomycete of origin is called Streptomyces avermilitis, and is quite unique in morphology as compared to other Streptomyces species. Chemically, Ivermectin, a macrocyclic lactone, is a 22,23 dihydro derivative of avermectin B_1.

The authors go on to describe the antiparasitic efficacy as broad spectrum against 2 major animal parasite phyla: Nemathelminthes and Arthropoda. It appears to be inactive against the phylum Platyhelminthes, due to the latter having neurophysiological differences. Nematodes covered include the superfamilies Trichostrongyloidea, Strongyloidea, Metastrongyloidea, Rhabditoidea, Ascaridoidea, Oxyuroidea, Spiruroidea, Filaroidea, and Trichuroidea. It is also extremely effective at lower than normal doses as compared to contemporary antiparasitic compounds.

The authors further describe the mode of action as blocking signal transmission between interneurons to excitatory motorneurons. The neurotransmitter GABA (gamma-amino-butyric-acid), the substance blocked, is normally used by nematodes and arthropods for this purpose. GABA functions to regulate chloride ion channels in crustucean muscles, so they feel the mode of action in nematodes and arthropods may be similar. In mammals, they conclude, GABA is found only in the central nervous system, protected by the blood-brain barrier, so doses of 40X

normal are required in cattle to produce toxicity and death.

The Ivermectin used in this study was under the trade name, "Ivomec," and is a 1% solution manufactured by MSD AGVET, Division of Merck & Co., Inc., Rahway, New Jersey. The package insert continues that it is in a 40% glycerol formal with propylene glycol, q.s. ad 100%.

Fecal Egg Counting

A vast majority of the time spent on this study was in the laboratory running fecal egg count procedures. This has continued to be the conventional method for qualitative and quantitative measurements of gastro-intestinal parasite numbers. A modification of the Wisconsin procedure was utilized which uses sugar flotation as outlined by Todd. The real key to this procedure is the preparation of the sugar solution with a specific gravity of 1.27 as outlined by Sloss (1970). She writes that this "Sheather" solution will float most of the eggs of commonly observed parasites.

METHODS

The first group of lambs was an intensively managed flock on continuous dry-lot in the Department of Animal and Range Science, South Dakota State University, Brookings, SD. The flock was served by a Suffolk ram for cross-bred lambs. The breeding ewes were in three groups: Finnish Targhee, Suffolk-Targhee, and straight Targhee.

Thirty ram and 30 ewe feeder lambs were randomly separated into three groups of 10 each and treated as follows:

Rams:	Ewes:
10 Ivermectin	10 Ivermectin
10 Levamisole	10 Levamisole
10 Control	10 Control

The dosage for Ivomec, the trade-name for the Merck and Co. compound, was 1 ml per 50 kg body weight as directed by label directions, given subcutaneously. The dosage for Levasole, the trade-name for the Pitman-Moore compound, was 2 ml per 100 lb body weight as directed by label directions, also given subcutaneously. The needles were 20 ga X 1 in.

Test No. I began on June 15, 1984, and terminated on July 18, 1984. All animals were weighed and fecal samples taken at the beginning and end of the test. See Table 1 for individual details on Test No. I. On July 23, 1984, a total of 15 ram lambs, 5 from each test group, were slaughtered at the Meat Science Department, South Dakota State University. Each set of viscera was laid out on a working table, and the ear tag removed from each animal and laid on the respective gut pile

to help ensure proper identification. The abomasum was located and removed from the rest of the viscera by excising it with surgical scissors at the juncture of the duodenum and of the omasum. Care was taken to not empty any of the abomasal contents until a 12 x 16 x 1 inch deep plastic tray was placed underneath. The abomasum was then cut open laterally, turned inside out, and washed out thoroughly with water to help flush any larvae or adult nematodes into the collecting tray. Extra water was added to the tray when necessary to suspend the nematodes, and the resulting supernatant carefully poured off. The nematodes, when found, were removed with a fine-pointed forceps, and placed in a glycerin-alcohol storing and clearing solution. Each animal's nematodes were separately stored for later identification.

Fecal samples from the test were taken back to the lab and egg counts performed via the Sheather flotation method as outlined by A. C. Todd, University of Wisconsin. A 2 gm fecal sample from each test animal was double centrifuged--the second time in a 1.27 specific gravity sugar solution--and eggs collected on a 22 mm square coverglass for viewing under the microscope.

The second group of lambs was a minimal-management flock of mixed breed (Western Whiteface) kept on pasture by the Veterinary Science Department, South Dakota State University. Sheep had been kept on this pasture continuously for many years, so it was well seeded with nematode eggs and larvae.

Forty-seven lambs from this group were weaned and randomly separated into two groups: half were treated with Ivermectin and the other half were controls. All animals ran together throughout the test.

Midway through the test, half of the treated animals were retreated with Ivermectin. This was done with the decision to continue the test for a longer period than previously intended.

This test began on July 2, 1984 and terminated on October 26, 1984. On September 4, 1984, the first weighback was performed, and the decision to continue the test made. On September 25, 1984, all animals were reweighed, and half of the treated animals were randomly chosen to receive a second injection of Ivermectin. Table 2 outlines the individual details of Test No. II. Fecal samples were taken on all 3 dates. On October 30, 1984, a total of 15 lambs were slaughtered: 5 had been treated twice, 5 had been treated once, and the other 5 head had been controls for the entire test. The viscera from each animal was explored as described in the first test and nematodes recovered.

The third aspect of this study began on January 23, 1985, and terminated on May 13, 1985. Blood samples were drawn at weekly intervals from the jugular vein of 6 animals: 2 bred ewes that were the dams of the lambs in Test No. II, 2 bred lambs that had received one injection with Ivermectin in the second test, and 2 bred lambs that had been control animals in the second test. Serum was recovered by centrifuging and was frozen for later lab work.

The work on the serum samples normally was resumed within 5 days. Serum samples were thawed out and mixed with HCl to activate the pepsinogen to pepsin. Samples were incubated at 37°C for 3 hours, along with one pooled sample that had trichloracetic acid (TCA) added to stop any digestion of plasma proteins from occurring. All samples were then

removed from the incubator, TCA was added to all remaining samples, and they were all centrifuged at 3000 rpm for 10 minutes. The supernatant was withdrawn and respun at 3000 rpm for 10 minutes to remove any further precipitated proteins. NaOH and Folin-ciocalteau reagent were added and allowed to react for at least 3 minutes, and the absorbance was read at 560 nm--visible spectrum--on a spectrophotometer. The absorbance values were applied to a mathematical formula to determine milliunits (mu) of tyrosine released by digestion of plasma proteins by pepsin. Table 3 outlines the plasma pepsinogen data.

Fecal samples were taken concurrently with the blood samples every week, and examined as previously outlined. The worm egg counts are also reflected in Table 3.

The final aspect of this study was the correlation of climatic data obtained from the Agricultural Engineering Department, South Dakota State University, with various factors of the study. Rainfall for the period April 1, 1984, through October 1, 1984, and temperature highs and lows for the period January 23, 1985, through May 13, 1985, were obtained. Tables 4 and 5 outline this data.

RESULTS

Nematodes Collected

Test No. I yielded no abomasal parasites, not even in the control animals. This is rare, and a tribute to the care these animals received.

Test No. II was a vastly different story. Unlike the lambs in the first test, these animals were on pasture instead of continuous dry-lot. We positively identified Haemonchus contortus, but found no other genera present. Table 3 summarizes the relative amount of burden to each animal, which shows little variation among the 3 groups. This necessitates statistical applications to give a true picture of the advantages of worming. Reinfection on pasture appears to be a rapid phenomenon.

The rainfall received in the spring of 1984 was around 5 inches above normal in Brookings County, and may have contributed to the thriving of Haemonchus as explained earlier.

Eggs Observed

Test No. I recovered a mere 2 eggs; this tends to agree with the observed absence of abomasal parasites.

Test No. II gave a wide variety of nematode eggs. The trichostrongylid eggs were not positively identified, owing to previously stated difficulties, but there were many other genera present including Nematodirus, Strongyloides, and Moniezia, the latter not being a nematode.

Statistical Applications

Test No. I showed no significant rate of weight gain for any of the groups at the 0.05 level.

Test No. II showed significant rate of gain increases for the second half of the study, following the second injection of Ivermectin, and in the animals that had received one injection only, at the end of the study. This was also at the 0.05 level.

Test No. III was analyzed to try to find a relationship between fecal egg numbers and milliunits of tyrosine. A linear regression model failed to show a significant regression in this test. This confirms the idea that plasma pepsinogen levels are caused by a source other than mature nematodes, and is more likely related to abomasal damage.

Environmental Factors

The "spring rise" was observed in the third test on May 1. The average temperature, however, had stayed above freezing starting on April 10, and the last freezing temperature was on April 26. This latter bit of information may be of consequence, but more must be understood about the mechanisms of resumed larval development in the spring.

Table 1. Amount of weight gained in 33 days by lambs utilized in Test No. I.

Female

Lamb No.	Initial Weight	Final Weight	Gain	Treatment
275	77 lb.	105 lb.	28 lb.	Ivomec[1]
136	91	114	23	Levasole[2]
098	85	104	19	Control
239	80	98	18	Control
160	87	105	18	Levasole
192	81	100	19	Ivomec
126	84	94	10	Ivomec
199	86	105	19	Levasole
175	97	116	19	Ivomec
194	80	106	26	Control
074	86	105	19	Levasole
150	95	110	15	Control
232	90	111	21	Ivomec
255	85	105	20	Ivomec
093	92	114	22	Levasole
137	88	103	15	Control
176	77	100	23	Levasole
235	94	114	20	Control
096	91	111	20	Ivomec
140	94	102	8	Control
161	90	110	20	Levasole

Table 1. (continued)

Lamb No.	Initial Weight	Final Weight	Gain	Treatment
082	95 lb.	116 lb.	21 lb.	Ivomec
084	92	105	13	Ivomec
172	95	118	23	Levasole
178	96	116	20	Levasole
046	86	100	14	Ivomec
045	85	100	15	Levasole
258	87	111	24	Control
222	85	105	20	Control
067	98	119	21	Control
Averages:	87.8	106.3	18.5	Ivomec
	88.5	107.7	19.2	Levasole
	88.6	107.2	18.6	Control
Males				
050	84	115	31	Ivomec
181	97	123	26	Control
110	90	115	25	Levasole
057	87	117	30	Ivomec
217	85	115	30	Levasole
102	105	138	33	Ivomec
270	94	125	31	Control
111	90	115	25	Levasole
135	95	131	36	Control

Table 1. (continued)

Lamb No.	Initial Weight	Final Weight	Gain	Treatment
202	85 lb.	116 lb.	31 lb.	Ivomec
282	94	127	33	Levasole
179	95	124	29	Control
272	83	120	37	Control
100	95	125	30	Levasole
223	95	128	33	Ivomec
070	93	120	27	Ivomec
252	99	129	30	Levasole
124	94	123	29	Control
170	93	120	27	Ivomec
162	89	121	32	Levasole
197	84	121	37	Control
231	80	96	16	Levasole
076	98	130	32	Ivomec
133	87	120	33	Control
169	94	120	26	Ivomec
209	94	117	23	Levasole
125	93	125	32	Control
155	95	115	20	Control
142	96	125	29	Levasole
230	76	96	20	Ivomec

Table 1. (continued)

Lamb No.	Initial Weight	Final Weight	Gain	Treatment
Averages:	91.0 lb.	120.0 lb.	29.0 lb.	Ivomec
	91.2	118.5	27.3	Levasole
	91.7	122.7	31.0	Control

[1]Ivomec is the registered trademark for Ivermectin by Merck and Co., Inc., Rahway, New Jersey.

[2]Levasole is the registered trademark for Levanisole by Pitman-Moore, Washington Crossing, New Jersey.

Table 2. Amount of weight gained by lambs utilized in Test No. II.

No.	Sex	7/2 Wt 1	9/4 Wt 2	9/25 Wt 3	10/26 Wt 4	7/2 Test 1	9/25 Test 2	(Gain) Wt 4-3	Wt 4-1	Wt 3-1
01	F	38 lb.	46 lb.	64 lb.	80 lb.	+	-	16 lb.	42 lb.	26 lbs.
02	F	38	48	58.5	73	-	-	14.5	35	20.5
03	F	43.5	60	75	88.5	+	+	13.5	45	31.5
04	M	31	(died 7/21/84)			-				
05	F	44	62	70.5	85	-	-	14.5	41	26.5
06	M	34.5	54.5	70.5	94	+	+	23.5	59.5	36
07	M	41	62	71.5	87.5	-	-	16	46.5	30.5
08	F	50.5	63	78	92	+	-	14	41.5	27.5
09	M	59	82	97	98	-	-	1	39	38
10	F	51.5	72	86.5	91.5	+	+	5	40	35
11	(tag not used)									
12	M	49	64	78	100	+	+	22	51	29
13	F	44	71.5	89	103	+	-	14	59	45
14	F	46.5	63.5	80	97.5	+	+	17.5	51	33.5
15	F	57	81.5	91	107	+	-	16	50	34
16	M	62	85	100	116	+	-	16	54	38
17	M	48	68	89.5	107	+	+	17.5	59	41.5
18	F	37.5	58.5	71	85	+	+	14	47.5	33.5
19	F	32.5	40	53.5	63	+	-	9.5	30.5	21
20	F	51	64	76	92.5	+	-	16.5	41.5	25
21	M	52.5	80.5	97	104.5	+	-	7.5	52	44.5

Table 2. (continued)

No.	Sex	7/2 Wt 1	9/4 Wt 2	9/25 Wt 3	10/26 Wt 4	7/2 Test 1	9/25 Test 2	(Gain) Wt 4-3	Wt 4-1	Wt 3-1
22	F	46.5	64.5	85.5	89	+	+	3.5	42.5	39
23	M	42.5	61	83	101	+	+	18	58.5	40.5
24	M	56	71	89.5	105	+	+	15.5	49	33.5
25	F	59.5	82	93	107	-	-	14	47.5	33.5
26	F	51	77	90	102.5	+	-	12.5	51.5	39
27	F	43	52.5	68.5	84	+	-	15.5	41	25.5
28	F	45	66	83.5	--[1]	-				38.5
29	F	41	42	(died 9/26/84)			-			
30	F	44	66	80.5	97	+	-	16.5	53	36.5
31	F	48.5	78	92	110	+	-	18	61.5	43.5
32	F	55	69.5	85	99.5	-	-	14.5	44.5	30
33	F	43	63	75.5	90.5	+	+	15	47.5	32.5
34	F	45	65.5	77	97.5	+	-	20.5	52.5	32
35	F	47	62	76	92.5	-	-	16.5	45.5	29
36	M	51	67	83.5	100	-	-	16.5	49	32.5
37	F	57	70.5	82	95.5	-	-	13.5	38.5	25
38	M	49	67	78.5	99	-	-	20.5	50	29.5
39	F	48.5	61	77.5	92	-	-	14.5	43.5	29
40	M	58.5	84	100.5	114.5	-	-	14	56	42
41	F	35	40	48	60.5	-	-	12.5	25.5	13

Table 2. (continued)

No.	Sex	7/2 Wt 1	9/4 Wt 2	9/25 Wt 3	10/26 Wt 4	7/2 Test 1	9/25 Test 2	(Gain) Wt 4-3	Wt 4-1	Wt 3-1
42	F	43	52.5	65	81.5	-	-	16.5	38.5	22
43	F	46	53	62	76	-	-	14	30	16
44	M	34	52.5	63	69.5	-	-	6.5	35.5	29
45	M	50	55	70	90	-	-	20	40	20
46	M	51.5	67	82.5	99	-	-	16.5	47.5	31
47	F	37	44	60.5	74	-	-	13.5	37	23.5
48	M	55	75	91	105	-	-	14	50	36

[1]Lamb 28 was not used in the second test.

Key: + = Received an injection of Ivermectin.
- = A control animal.

Table 3. Summary of plasma pepsinogen values and fecal egg counts in Test No. III.

Date	Ewe No.	mu tyrosine	Trichostrongylid eggs per gm
1/23	6[4]	n/a[1]	n/a
	10[4]	0[2]	n/a
	40[5]	240	21
	41[5]	320	39
	42[6]	n/a	n/a
	43[6]	80	18
1/30	6	0	4
	10	0	39
	40	120	0
	41	280	43
	42	180	2
	43	140	8
2/6	6	40	1
	10	600	39
	40	460	0
	41	340	8
	42	320	2
	43	260	12
2/20	6	20	2
	10	0	31
	40	200	2
	41	60	13
	42	980	0
	43	140	1
2/27	6	140	2
	10	160	15
	40	320	0
	41	220	68
	42	460	15
	43	320	16
3/13	6	60	15
	10	0	4
	40	160	9
	41	980	163
	42	1640	3
	43	1920	46

Table 3. (Continued)

Date	Ewe No.	mu tyrosine	Trichostrongylid eggs per gm
3/20	6	20	0
	10	80	0
	40	240	2
	41	340	1
	42	320	1
	43	180	0
3/27	6	40	6
	10	120	24
	40	200	2
	41	120	106
	42	320	0
	43	200	31
4/3	6	260	0
	10	1300	0
	40	440	8
	41	560	79
	42	420	3
	43	280	10
4/17	6	40	12
	10	260	22
	40	160	77
	41	320	30
	42	160	7
	43	160	10
5/1	6	n/a	340
	10	n/a	141
	40	n/a	12
	41	n/a	305
	42	n/a	71
	43	n/a	158

Table 3. (Continued)

Date	Ewe No.	mu tyrosine	Trichostrongylid eggs per gm
5/13	6	200	600[3]
	10	220	600[3]
	40	220	213
	41	340	71
	42	400	69
	43	340	56

[1]Values were not available.

[2]Values that calculated to less than 0 were reported as 0.

[3]Estimated.

[4]These were ewes.

[5]These were control ewe-lambs.

[6]These ewe-lambs had received one injection.

Table 4. Rainfall data for the period April 1, 1984, through October 1, 1984, as compared to a 30-year average: 1951-1980: Brookings County

Month	1984	1951-1980	Difference
April	3.38 in	2.02 in	+1.36 in
May	3.06	3.06	0
June	8.48	4.41	+4.07
July	2.17	2.85	-0.68
August	1.64	3.15	-1.51
September	1.83	1.95	-0.12
Totals	20.56	17.44	+3.12

Table 5. Temperature highs, lows, and means for the period January 23, 1985, through May 13, 1985

	Temperature °F				Temperature °F				Temperature °F		
	Max	Min	Mean		Max	Min	Mean		Max	Min	Mean
Jan				Mar				Apr			
23	20	5	12.5	1	54	30	42.0	7	45	26	35.5
24	28	14	21.0	2	45	21	33.0	8	46	20	33.0
25	31	-13	9.0	3	26	16	21.0	9	41	21	31.0
26	10	-11	- 0.5	4	21	2	11.5	10	59	28	43.5
27	27	9	18.0	5	31	11	21.0	11	67	45	56.0
28	17	-14	1.5	6	38	13	25.5	12	74	43	58.5
29	17	-10	3.5	7	38	19	28.5	13	72	36	54.0
30	24	-15	4.5	8	35	18	26.5	14	38	33	35.5
31	-11	-22	-16.5	9	37	19	28.0	15	67	32	49.5
				10	43	19	31.0	16	71	40	55.5
Feb				11	46	30	38.0	17	64	41	52.5
1	- 2	-21	-11.5	12	38	20	29.0	18	87	40	63.5
2	- 4	-23	-13.5	13	38	21	29.5	19	90	54	72.0
3	9	-23	- 7.0	14	38	23	30.5	20	82	53	67.5
4	1	-17	- 8.0	15	40	30	35.0	21	69	43	56.0
5	3	-12	- 4.5	16	53	27	40.0	22	80	49	64.5
6	7	-18	- 5.5	17	42	26	34.0	23	54	45	49.5
7	0	-19	- 9.5	18	50	31	40.5	24	54	33	43.5
8	8	- 8	0	19	54	29	41.5	25	60	34	47.0
9	20	10	15.0	20	54	30	42.0	26	57	32	44.5
10	23	3	13.0	21	56	34	45.0	27	45	33	39.0
11	10	-15	- 2.5	22	59	33	46.0	28	61	35	48.0
12	12	-14	- 1.0	23	52	31	41.5	29	69	46	57.5
13	19	0	9.5	24	46	32	39.0	30	74	54	64.0
14	17	- 2	7.5	25	37	33	35.0				
15	18	- 1	8.5	26	52	40	46.0	May			
16	34	7	20.5	27	61	30	45.5	1	66	40	53.0
17	35	12	23.5	28	45	32	38.5	2	66	36	51.0
18	39	18	28.5	29	54	27	40.5	3	74	45	59.5
19	30	11	20.5	30	31	27	29.0	4	77	58	67.5
20	40	25	32.5	31	32	27	29.5	5	80	42	61.0
21	49	30	39.5					6	70	37	53.5
22	35	23	29.0	Apr				7	73	41	57.0
23	45	28	36.5	1	40	25	32.5	8	69	40	54.5
24	33	24	28.5	2	50	29	39.5	9	82	43	62.5
25	40	25	32.5	3	63	29	46.0	10	86	62	74.0
26	50	17	33.5	4	52	33	42.5	11	83	59	71.0
27	24	16	20.0	5	47	29	38.0	12	79	51	65.0
28	44	24	34.0	6	42	24	33.0	13	65	37	51.0

Table 6. Nematode burdens of slaughtered lambs in Test No. II

Treatment	Lamb Number	Burden
Ivomec-Ivomec	3	**
	10	0
	12	****
	14	***
	22	0
Ivomec-Control	8	*
	13	**
	20	****
	30	***
	34	****
Control-Control	5	*
	7	***
	37	****
	38	***
	39	****

Key: 0 None
* Very Light -- less than 10.
** Light
*** Heavy
**** Very Heavy -- > 1000.

SUMMARY

Sheep can become heavily infected with internal parasites. The nematode family Trichostrongylidae is one such group, found primarily in the abomasum.

Traditional methods for detecting internal parasites utilizes the fecal egg count, but this method tends to lose its accuracy when the L_4--fourth stage larvae-- go "hypobiotic" in the fall. This phenomenon may be similar to diapause in insects.

The plasma pepsinogen test is used to detect abomasal damage, and some researchers are testing its value as a diagnostic tool to predict relative number of nematode parasites in a "hypobiotic" state. We found no statistical relationship between trichostrongylid egg numbers and plasma pepsinogen levels in bred lambs and bred ewes.

We did observe the "spring rise" which is when the nematode parasites show a significant increase in fecal egg numbers, occurring near lambing time.

We also observed a significant increase in the rate of gain of lambs on pasture when injected with Ivermectin, a new antiparasitic compound that eliminates L_4 from abomasal glands. A similar, but shorter test on dry-lot lambs showed no significant increase in rate of gain utilizing Ivermectin or with another compound, Levamisole.

The only trichostrongylid positively identified was Haemonchus contortus, and it is felt that their solo presence and great numbers were due to the unusually wet spring encountered in 1984.

LITERATURE CITED

Anderson, J., J. Armour, W. F. H. Jarrett, F. W. Jennings, J. S. D. Ritchie, and G. M. Urquhart. 1965. A field study of parasitic gastritis in cattle. The Veterinary Record. 77(10):1196-1204.

Armour, J. 1974. Parasitic gastroenteritis in cattle. The Veterinary Record. 95(10):391-395.

Black, H. 1973. As told to Hazel Shave of the S. D. S. U. Veterinary Diagnostic Lab.

Campbell, W. C., M. H. Fisher, E. O. Stapley, G. Albers-Schonberg, and T. A. Jacob. 1983. Ivermectin: a potent new antiparasitic agent. Science. 221(8):823-828.

Chiejina, S. N., and F. G. Clegg. 1978. Some observations on the epidemiology of ostertagiasis in calves in Britain: an analysis of laboratory diagnostic and field data for 1974 to 1976. British Veterinary Journal. 134:541-550.

Corwin, R. M., and M. A. Brauer. 1984. The Control of Internal Parasites. American Hoechst Corporation. 16 pp.

Dawes, B. 1976. Advances in Parasitology. Academic Press, London, England. 462 pp.

Dunn, A. M. 1978. Veterinary Helminthology. William Heinemann Medical Books Limited, London, England. 323 pp.

Edwards, K., R. P. Jepson, and K. F. Wood. 1960. Value of plasma pepsinogen estimation. British Medical Journal. 240(1):30-32.

Georgi, J. R. 1969. Parasitology for Veterinarians. W. B. Saunders Co., Philadelphia, Pennsylvania. 237 pp.

Harvey-White, J. D., and E. H. Allen. 1982. Method for determining serum pepsinogen concentration, using pepsin standards and ultraviolet absorbance. American Journal of Veterinary Research. 43(3):1317-1320.

Harvey-White, J. D., J. P. Smith, E. Parbuoni, and E. H. Allen. 1983. Reference serum pepsinogen concentrations in dairy cattle. American Journal of Veterinary Research. 44(3):115-116.

Hickman, C. P., C. P. Hickman, F. M. Hickman, and L. S. Roberts. 1979. Integrated Principles of Zoology. C. V. Mosby Co., St. Louis, Missouri. 1086 pp.

Highnam, E. C., and L. Hill. 1977. The Comparative Endocrinology of the Invertebrates. William Clowes & Sons, Limited, London, England. 357 pp.

Honess, R. F., and K. B. Winter. 1956. Diseases of Wildlife in Wyoming. Wyoming Game and Fish Commission. 279 pp.

Ivens, V. R., D. L. Mark, and N. D. Levine. 1978. Principal Parasites of Domestic Animals in the United States. University of Illinois, Urbana-Champaign, Illinois, 270 pp.

Johnstone, C. 1985. Dairy cattle and their worms. Animal Nutrition and Health. (3):25-31.

Malone, J. B. 1983. Research needs and priorities for ruminant parasites in the United States. American Journal of Veterinary Research. 44:1836-1846.

Miller, H. L. 1974. Common sheep parasites. Cooperative Extension Service, South Dakota State University. No. FS 373. 6 pp.

Morgan, B. B., and P. A. Hawkins. 1949. Veterinary Helminthology. Burgess Publishing Co., Minneapolis, Minnesota. 400 pp.

Ransom, B. H. 1908. The prevention of losses among sheep from stomach worms (Haemonchus contortus). U.S. Department of Agriculture, Bureau of Animal Industry. No. 157. 10 pp.

Selman, I. E., J. Armour, and F. W. Jennings. 1977. Interpretation of the plasma pepsinogen test. The Veterinary Record. 100(3): 249-250.

Sloss, M. W. 1970. Veterinary Clinical Parasitology. Iowa State University Press, Ames, Iowa. 250 pp.

Thienpont, D., F. Rochette, and O. F. J. Vanparijs. 1979. Diagnosing Helminthiasis through Coprological Examination. Beerse, Belgium, 187 pp.

Thomas, R. J., and P. J. Waller. 1975. Significance of serum pepsinogen and abomasal pH levels in a field infection of O. circumcincta in lambs. The Veterinary Record. 97(12):468-471.

Todd, A. C. Wisconsin procedure for fecal worm egg counts by a sugar flotation method. Reprint by Merck & Co., Inc., Rahway, New Jersey. No. 81-861-41R 1080.

U.S. Department of Agriculture: Sheep and Goats. LvGnl (1-80). 1980. Crop Reporting Board, Washington, D.C.

Vander, A. J., J. H. Sherman, D. S. Luciano. 1980. Human Physiology: The Mechanisms of Body Function. McGraw-Hill Book Co., New York, New York. 724 pp.

Whitlock, J. H. 1960. Veterinary Parasitisms. Lea & Febiger, Philadelphia, Pennsylvania. 236 pp.